Bull. Fac. Agr., Saga Univ. No. 83 : 59~69 (1998)

Impact of Cholesterol on Liver Microsomal Phospholipid Metabolism in Rats Fed a Diet Containing Fish Oil or Beef Tallow

Jae-Young CHA and Teruyoshi YANAGITA
(Laboratory of Nutrition Biochemistry)
Received September 7, 1998

Summary

The effect of dietary cholesterol on phospholipid metabolism and on the fatty acid composition of the phosphatidylcholine and phosphatidylethanolamine fractions in liver microsomes were studied in rats fed a diet containing fish oil (FO) or beef tallow (BT). The liver contents of triglyceride and cholesterol in the cholesterol-free FO and BT diet groups did not differ significantly, while the liver phospholipid content was higher in the FO group than in the BT group. Plasma lipid concentrations in rats fed a cholesterol-free diet were lower in the FO group than in the BT group. After cholesterol supplementation, liver phospholipid content decreased whereas liver contents of triglyceride and cholesterol increased significantly in both groups. Plasma concentrations of phospholipid and triglyceride increased after dietary cholesterol supplementation in both groups, while cholesterol decreased only moderately in the FO group. Dietary cholesterol affected liver microsomal phospholipids: the proportion of phosphatidylcholine decreased in the FO group, and the proportion of phosphatidylcholine also decreased slightly at the expense of phosphatidylethanolamine in the BT group. The main component of highly unsaturated fatty acids in microsomal phosphatidylcholine and phosphatidylethanolamine was docosahexaenoic acid (22:6 n-3) in the FO group, and arachidonic acid (20:4 n-6) in the BT group. Supplementation of both diets with cholesterol increased the proportion of arachidonic acid at the expense of stearic acid (18:0) in phosphatidylcholine and decreased the proportion of docosahexaenoic acid in phosphatidylethanolamine. The activity of CTP: phosphocholine cytidylyltransferase, the rate-limiting enzyme of phosphatidylcholine synthesis, was increased in liver microsomes, whereas a decrease of the enzyme activity was found in the cytosol of both groups upon adding cholesterol to the diets. In conclusion, the results indicate that dietary cholesterol profoundly influences phospholipid metabolism in the rat.

Key words: Cholesterol, Phospholipid, Fish oil, Beef tallow, CTP: phosphocholine cytidylyltransferase.

Introduction

Changes in membrane fluidity caused by altering the cholesterol/phospholipid ratio can cause alterations in specific cholesterol-phospholipid interactions or modifications in phospholipid composition. These are some of the explanations for the cholesterol effect in

biological membrane functions[1]. The modulation of some membrane-associated enzyme activities and some membrane receptor functions could be influenced when cholesterol content is varied or when the molecular composition of phospholipid is changed[1]. When cholesterol is added to the diet, liver cholesterol and triglyceride contents increase, and very-low-density lipoprotein (VLDL) secretion also increases, which suggests that cholesterol is required for VLDL formation[2-4]. Also inhibition of phospholipid synthesis has been shown to decrease VLDL secretion, which can cause accumulation of triglyceride in the liver[5]. It was postulated that alterations of phospholipid metabolism may play an important role in the formation of hepatic lipoproteins. CTP: phosphocholine cytidylyltransferase (CT), which converts phosphorylcholine into CDP-choline, is the rate-limiting enzyme in phosphatidylcholine synthesis[6,7]. Phosphatidylcholine synthesized by this pathway appears to be involved in the secretion of VLDL[6,8]. It is also reported that cholesterol feeding stimulates the membrane-bound CT activity in rat liver[4]. Hepatocytes isolated from rats fed a choline-deficient diet are reported to have reduced secretion of VLDL, but not of high-density lipoprotein[9]. It is therefore important to test whether dietary cholesterol can influence enzyme activities and the phospholipid metabolism and distribution in the hepatic cell membranes in animals fed with various fat-containing diets.

The ingestion of fish oil containing n-3 polyunsaturated fatty acids has been found to reduce plasma triglyceride levels in human subjects and experimental animals[10-13]. It is known that dietary lipids play a significant role in determining plasma concentrations of triglyceride and cholesterol[10,11,14] and that increasing these two lipids above normal levels is atherogenic[15]. Feeding rats with different fats affects the fatty acid composition of microsomal phospholipids and the synthesis of phospholipid in the liver[16,17]. While many of these studies were related to plasma lipid concentration, some studies focused on the effects of dietary cholesterol on lipid metabolism in the liver[17,18]. The present study was undertaken to elucidate how dietary cholesterol affects phospholipid metabolism in rats fed with various fatty acid diets.

Materials and Methods

Animals and diets. Male Sprague-Dawley rats (four weeks old) were purchased from Kyudo Experimental Animals (Tosu, Japan) and acclimated in a room maintained at 20-23 °C with a 12-h light-dark cycle. Before starting the experiment, rats were allowed free access to a commercial chow diet. Then rats were divided into four groups of six animals each according to the source of the dietary fats. The diets were prepared according to recommendations of the American Institute of Nutrition[19] and contained (by wt%) casein 20, fat 10, vitamin mixture (AIN-76) 1, mineral mixture (AIN-76) 3.5, choline chloride 0.15, cellulose powder 4, and sucrose to make 100%. The rats were fed on diets with (for cholesterol supplementation group) or without (for control group) 0.5% (w/w) cholesterol and 0.125% (w/w) cholic acid at the expense of sucrose. The fatty acid composition of the dietary fats is shown in Table 1. The rats were fed these diets *ad libitum* for 14 days. At the end of the feeding period, they were killed by decapitation, and the liver was excised

immediately.

Lipid analysis. Total liver lipids were extracted by the method of Folch et al.[20]. The concentrations of liver cholesterol and triglyceride were measured by the methods of Sperry and Webb[21] and Fletcher[22], respectively. Phospholipid as quantified by phosphorus content was measured by the method of Rouster et al.[23]. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine plus phosphatidylinositol , lysophosphatidylcholine, sphingomyelin and phosphatidic acid were separated by thin layer chromatography using chloroform/methanol/water/acetic acid (25:15:4:2, by vol) as the developing solvent[24]. The distribution of phospholipid classes was determined by their phosphorus content[23]. The fatty acid composition of liver microsomal phosphatidylcholine and phosphatidylethanolamine was determined by gas-liquid chromatography (Shimadzu GC-14 equiped with flame ion detector and capillary column Omega Wax, 0.25 mm X 30 m, Supelco, USA) after transmethylation with HCl-methanol[25]. The concentrations of serum cholesterol, triglyceride and phospholipid were assayed enzymatically with commercial kits (Wako Pure Chemical Ind., Osaka, Japan) according to the protocol supplied by the manufacturer.

Table 1. Fatty acid composition of dietary fats.

	Beef tallow	Fish oil
	(Weight %)	
14 : 0	4.3	6.9
16 : 0	32.0	8.1
16 : 1	4.1	10.2
18 : 0	19.1	2.1
18 : 1	36.1	11.8
18 : 2	2.3	6.6
18 : 3	0.0	2.9
20 : 5	0.0	28.1
22 : 6	0.0	11.4
SFA	55.4	26.8
MUFA	40.2	22.0
PUFA (n-6)	2.3	6.6
PUFA (n-3)	0.0	42.4

Abbreviations : SFA, saturated fatty acid
MUFA, monounsaturated fatty acid
PUFA, polyunsaturated fatty acid.

Analytical methods. Liver microsomal and cytosolic fractions were prepared as described previously[24] and stored at -80 °C. Protein was assayed by the method of Lowry et al.[26] using bovine serum albumin as a standard. The enzymatic activity of CTP: phosphocholine cytidylyltransferase was measured by the procedure described by Wright et al.[27] using phospho [methyl-^{14} C] choline as a substrate; 1 mM of phosphatidylcholine-oleate was added to the assay mixture to measure the activity of cytosolic CTP: phosphocholine cytidylyltransferase. The activity of choline kinase in the liver cytosol was measured using [methyl-^{14} C] choline as a substrate by the procedure described previously[24,28].

Statistical analysis. Data were analyzed by Duncan's new multiple-range test[29]. Values are expressed as mean ± SE. Values marked with different letters in the figures are significantly different at $p < 0.05$.

Results

Liver and plasma lipids. As shown in Fig. 1, the liver contents of triglyceride and cholesterol were not significantly different in the FO and BT cholesterol-free diet groups, while the liver phospholipid content was higher in the FO group than in the BT group. Cholesterol supplementation reduced liver phospholipid content and significantly increased

liver triglyceride and cholesterol contents in both groups. As shown in Fig. 2, in animals fed a cholesterol-free diet, plasma concentrations of phospholipid, triglyceride, and cholesterol were lower in the FO group than in the BT group. After dietary cholesterol supplementation, plasma concentrations of phospholipid and cholesterol increased and that of plasma triglyceride decreased significantly in the BT group. In contrast, the plasma concentration of phospholipid increased whereas that of cholesterol decreased moderately in rats fed a cholesterol diet in the FO group.

Microsomal phospholipid distribution. As shown in Table 2, the phospholipid distribution of liver microsomal membranes was modulated differently after dietary cholesterol supplementation in the two groups. In the BT group, the proportion of phosphatidylcholine decreased slightly, while the proportion of phosphatidylethanolamine increased modestly after dietary cholesterol supplementation in both groups, and while the proportion of

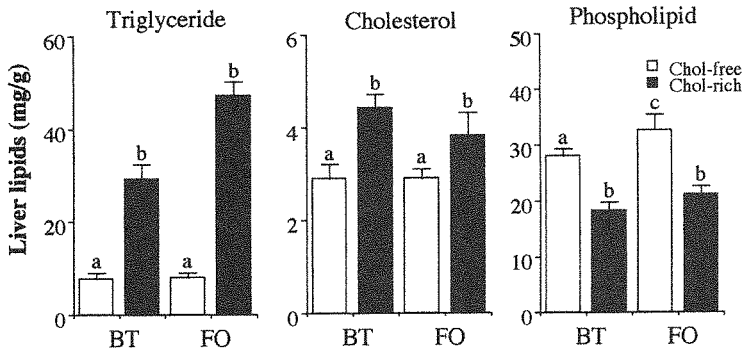

Fig. 1 The contents of triglyceride, cholesterol, and phospholipid in livers of rats fed a diet containing beef tallow (BT) or fish oil (FO) with or without supplementation of cholesterol.
Values are given as the means ± SE of 6 rats. Between the groups, values with different letters are significantly different at $p < 0.05$.

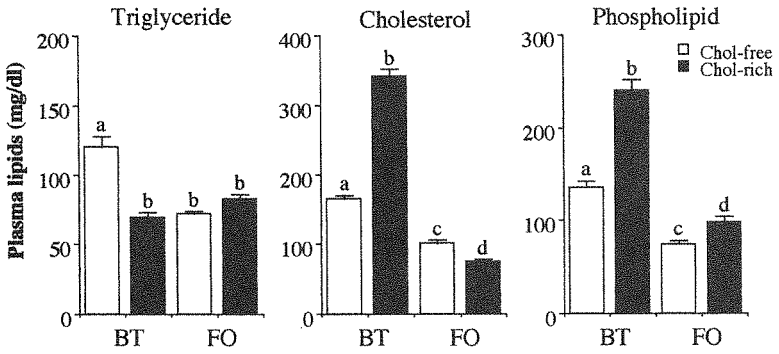

Fig. 2 The contents of triglyceride, cholesterol, and phospholipid in plasma of rats fed a diet containing beef tallow (BT) or fish oil (FO) with or without supplementation of cholesterol.
Values are given as the means ± SE of 6 rats. Between the groups, values with different letters are significantly different at $p < 0.05$.

Table 2. Phospholipid distributions in liver microsomes of rats fed a diet containing beef tallow or fish oil with or without supplementation of cholesterol.

	Beef tallow		Fish oil	
	Chol-free	Chol-rich	Chol-free	Chol-rich
	(% of total phospholipid)			
PC	65.1±0.7	61.9±0.3	60.7±0.3	53.6±1.9
PE	14.7±0.3	17.5±0.4	19.4±0.1	18.8±2.8
PS+PI	13.3±0.7	14.3±0.2	13.3±0.2	14.3±1.4
SPM	3.54±0.3	2.87±0.5	3.59±0.3	5.45±0.8
LysoPC	2.24±0.1	2.44±0.2	2.03±0.1	5.57±1.6
PA	1.17±0.7	0.97±0.2	1.07±0.7	2.25±0.6
PC/PE	4.42	3.53	3.12	2.85

Rats were fed semipurified diets containing beef tallow or fish oil with or without supplementation of cholesterol. Values are given as the means ± SE of 6 rats. LPC: lysophosphatidylcholine, SPM: sphingomyelin, PC: phosphatidylcholine, PS+PI: phosphatidylserine+phosphatidylinositol, PE: phosphatidylethanolamine, PA: phosphatidic acid.

sphingomyelin and phosphatidic acid decreased. In the FO group, the proportion of phosphatidylcholine decreased upon cholesterol supplementation, while that of phosphatidylethanolamine showed almost no change. In addition, the proportions of sphingomyelin, lysophosphatidylcholine and phosphatidic acid increased upon cholesterol supplementation, while the proportion of acidic phospholipids, phosphatidylserine plus phoshatidylinositol, was unchanged.

Activities of liver enzymes related to phospholipid synthesis. As shown in Fig. 3, the cytosolic activity of choline kinase, the first enzyme in the pathway of phosphatidylcholine formation, was reduced slightly after dietary cholesterol supplementation in both groups. However, the activity of CT, which catalyzes CDP-choline formation from phosphocholine,

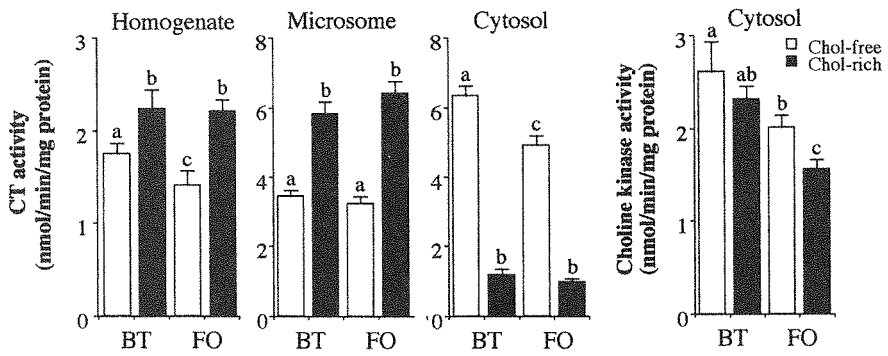

Fig. 3 The activities of CTP : phosphorylcholine cytidylyltransferase (CT) and choline kinase in livers of rats fed a diet containing beef tallow (BT) or fish oil (FO) with or without supplementation of cholesterol.
Values are given as the means ± SE of 6 rats. Between the groups, values with different letters are significantly different at $p < 0.05$.

was increased in microsomes and decreased in the cytosol after dietary cholesterol supplementation in both groups. These concomitant translocations of CT activities from cytosol to microsome were shown in both groups after adding cholesterol to the diets. The incrementation of microsomal CT activity was correlated with the increased activity in liver homogenates. However, the extent of the increase of CT activity was higher in the FO group (60%) than in the BT group (34%).

Fatty acid composition of liver microsomal phospholipids. As shown in Table 3. cholesterol supplementation affected differently the distribution of fatty acids in phosphatidylcholine and phosphatidylethanolamine molecules, especially for arachidonic acid, docosahexaenoic acid and stearic acid. The distribution of saturated fatty acid in phosphatidylcholine decreased moderately after dietary cholesterol supplementation in both groups, while it decreased in phosphatidylethanolamine only in the FO group upon cholesterol supplementation. The distribution of oleic acid in phosphatidylcholine and phosphatidylethanolamine increased upon addition of dietary cholesterol in both the BT and the FO groups, but the effect was more profound in the FO group. The arachidonic acid content was greatly increased in the phosphatidylcholine fraction in both the BT and the FO groups. In the phosphatidylethanolamine fraction, the proportion of arachidonic acid was decreased in the BT group while it was increased in the FO group. The distribution of docosahexaenoic acid in phosphatidylcholine was increased moderately in both the BT and the FO groups. In the phosphatidylethanolamine fraction, the distribution of docosa-

Table 3. Fatty acid compositions in the liver microsomal phosphatidylcholine and phosphatidylethanolamine of rats fed a diet containing beef tallow or fish oil with or without supplementation of cholesterol.

	Beef tallow		Fish oil	
	Chol-free	Chol-rich	Chol-free	Chol-rich
Phosphatidylcholine (% of total fatty acid)				
16 : 0	27.3±1.2	22.5±0.4	38.2±0.1	27.8±1.6
16 : 1	3.9±0.4	4.5±0.1	5.5±0.1	5.9±0.2
18 : 0	23.1±1.1	19.9±0.1	18.0±0.1	10.5±0.7
18 : 1	20.8±1.1	23.8±0.2	12.9±0.1	15.7±0.4
18 : 2 n-6	5.9±0.1	9.3±0.1	1.9±0.1	3.1±0.3
20 : 4 n-6	8.6±1.7	13.3±3.3	5.6±0.1	8.0±0.5
20 : 5 n-3	n.d.	n.d.	5.3±0.2	9.4±0.4
22 : 6 n-3	3.5±1.3	3.9±0.1	6.4±0.2	8.8±1.2
Phosphatidylethanolamine (% of total fatty acid)				
16 : 0	20.1±0.3	19.6±1.8	25.5±0.8	21.4±2.2
16 : 1	1.5±0.1	0.7±0.1	1.3±0.1	0.7±0.2
18 : 0	24.7±0.5	25.9±1.8	23.5±0.9	22.9±2.2
18 : 1	9.6±0.6	13.1±0.3	4.6±0.3	9.2±0.2
18 : 2 n-6	2.0±0.1	n.d.	2.7±0.2	1.0±0.2
20 : 4 n-6	18.9±0.2	16.8±1.9	6.3±0.1	6.8±0.8
20 : 5 n-3	n.d.	n.d.	n.d.	7.7±0.7
22 : 6 n-3	13.9±1.8	8.4±1.6	23.2±1.85	18.2±1.1

Values are given as the means ± SE of 6 rats. n.d. : not detected.

hexaenoic acid was decreased.

Discussion

The present study investigated the effects of cholesterol supplement of fish oil or beef tallow diets on phospholipid metabolism in rat liver microsomes. In animals fed a cholesterol-free diet, the liver lipid contents in the two groups did not differ significantly. Cholesterol supplementation decreased the liver phospholipid content, while the liver contents of triglyceride and cholesterol increased, especially in the FO group (Fig. 1). Fungwe et al.[3] and Lie et al.[29] reported that dietary cholesterol increased the synthesis and the mass of triglyceride and cholesteryl ester in the liver, concomitant with an increase in the secretion of VLDL-lipids. They also observed that cholesterol feeding reduced the activity of carnitine palmitoyltransferase, which may lead to cholesterol and triglyceride accumulation by increasing the substrate supply. However, the hepatic phospholipid content was decreased in the cholesterol-fed rats[30], which may lead to impairment of the membrane functions in the liver.

The increase in the amounts of liver cholesterol upon cholesterol supplement of the diet may modify membrane fluidity. Consequent alterations in the activities of membrane -bound enzymes may influence the phospholipid metabolism in the liver. Phosphatidylcholine is a major component of phospholipids in biological membranes and VLDL[6,8,31]. Phosphatidylcholine is synthesized mainly througth the CDP-choline pathway, catalyzed by choline kinase, CTP: cholinephosphate cytidylyltransferase and cholinephosphate transferase[6,7,32]. Newly synthesized phosphatidylcholine is reported to be required for the synthesis and secretion of VLDL in hepatocytes[6,8]. Thus, alteration of phosphatidylcholine biosynthesis may influence lipoprotein metabolism. Because choline kinase and CT are reported to be rate-limiting enzymes for phosphatidylcholine biosynthesis[6,7], we evaluated the effect of cholesterol-feeding on these enzyme activities. Our results showed that the activity of choline kinase in the liver cytosol was decreased slightly after dietary cholesterol supplement in both groups (Fig. 3). The activity of CT was increased in microsomes and decreased in the cytosol after dietary cholesterol supplement in both groups (Fig. 3). Lim et al. reported that rats fed a diet enriched in cholesterol (5%) and cholate (2%) displayed 2-fold greater translocation of cytosol CT to microsomes than control animals[4]. This phenomenon suggests an altered subcellular distribution of CT in rats fed a cholesterol-supplemented diet. It is thought that CT activity is regulated by translocation from the cytosolic inactive form to the microsomal active form[33-35]. The possibility that this alteration of the subcelluar distributions of CT was due to a decrease in the level of phosphatidylcholine in the microsomal membranes should be tested. Other potential mechanisms for CT translocation, such as changes in the supply of fatty acids, may also be considered.

The present study showed that the amount of liver phospholipid decreased and that of liver cholesterol increased after cholesterol supplementation in both dietary groups (Fig.

1); therefore the cholesterol/phospholipid ratio increased two-fold. The phospholipid compositions (Table 2) and the fatty acid moieties of phosphatidylcholine and phosphatidylethanolamine (Table 3) in microsomal preparations were also studied. When rats were fed cholesterol-free diets, the BT group contained more phosphatidylcholine and less phosphatidylethanolamine than the FO group. The proportion of phosphatidylcholine decreased after dietary cholesterol supplement in both groups. On the other hand, phosphatidylethanolamine in the BT group increased and phosphatidylethanolamine in the FO group decreased, resulting in the reduction of the ratio of phosphatidylcholine/phosphatidylethanolamine in both groups upon cholesterol supplement. The microsomal CT activity and phosphatidylcholine content were not changed. These results suggest that phosphatidylcholine catabolism may be enhanced upon cholesterol supplement only in the FO group.

The main component of highly unsaturated fatty acids in liver microsomes of the FO group was docosahexaenoic acid in phosphatidylcholine and phosphatidylethanolamine, while in the BT group it was arachidonic acid in phosphatidylcholine and phosphatidylethanolamine. Supplementation of the BT or the FO diet with cholesterol resulted in an increase of the proportion of arachidonic acid and a decrease of the proportion of stearic acid in phosphatidylcholine. By contrast, the same dietary supplementation resulted in a decrease of docosahexaenoic acid in phosphatidylethanolamine. Thus, the effects of cholesterol differed according to the dietary fatty acid type.

The secretion of phospholipid increased significantly after dietary cholesterol supplementation in both groups (Fig. 2), in agreement with the previous reports[4,18,36]. Ohtani et al. reported that VLDL secretion was stimulated in cultured hepatocytes from cholesterol-fed hamsters only when fat was included in the diet[9]. Our data and previous reports suggest that dietary cholesterol may stimulate the secretion of VLDL. A positive correlation between cholesterol and phospholipid levels in plasma was noted, in agreement with previous reports[3,30]. The present study showed a decrease in the content of plasma lipids in rats fed fish oil, which may be the result of inhibiting the synthesis/secretion of VLDL in the liver[10-13]. In conclusion, the impact of dietary cholesterol upon phospholipid metabolism in the rat differs according to the source of the dietary fat.

References

1. Tilcock, C. P., M. B. Bally, S. B. Farren, and P. R. Cullis (1982) Influence of cholesterol in the structural preferences of dioleoylphosphatidylethanolamine-dioleoylphosphatidylcholine systems: A phosphorus-31 and deuterium magnetic resonance study. *Biochemistry* **21**, 4596-4601.
2. Khan, B. K., H. G. Wilcox, and M. Heimberg (1989) Cholesterol is required for secretion of very-low-density lipoprotein by rat liver. *Biochem.* J. **259**, 807-816.
3. Fungwe, T.V., L. M. Cagen, C.G. Cook, H.G. Wilcox, and M. Heimberg (1993) Dietary cholesterol stimulates hepatic biosynthesis of triglyceride and reduces oxidation of fatty acids in the rat. *J. Lipid Res.* **34**, 933-941.
4. Lim, P. H., P. H. Pritchard, H. B. Paddon, and D. E. Vance (1983) Stimulation of hepatic phosphatidylcholine biosynthesis in rats fed a high cholesterol and cholate diet correlates with

translocation of CTP: phosphocholine cytidylyltransferase from cytosol to microsomes. *Biochim. Biophys. Acta* **753**, 74-82.

5. Vance, E. J. and D. E. Vance (1985) The role of phosphatidylcholine biosynthesis in the secretion of lipoprotein from hepatocytes. *Can. J. Biochem. Cell. Biol.* **63**, 870-881.
6. Vance, J. E., and D. E. Vance (1986) Specific pools of phospholipids are used for lipoprotein secretion by cultured rat hepatocytes. *J. Biol. Chem.* **261**, 4486-4491.
7. Yanagita, T., K. Yamamoto, T. Ide, and N. Enomoto (1990) Effect of choline deficiency on CTP: phosphoylcholine cytidylyltransferase and choline kinase activities in rat liver subcellular fractions. *J. Nutr. Sci. Vitaminol.* **36**, 287-290.
8. Vance, J. E., and D. E. Vance (1988) Does rat liver golgi have the capacity to synthesize phospholipids for lipoprotein secretion? *J. Biol. Chem.* **263**, 5898-5909.
9. Ohatani, H., K. hayashi, Y. Hirata, S. Dojo, K. Nukachima, E. Nishio, H. Kurushima, M. Saeki, and G. Kajiyama (1990) Effects of dietary cholesterol and fatty acids plasma cholesterol level and hepatic lipoprotein metabolism. *J. Lipid Res.* **31**, 1413-1422.
10. Ikeda, I., J.-Y. Cha, T. Yanagita, N. Nakatani, K. Oogami, K. Imaizumi, and K. Yazawa (1998) Effects of dietary α-linolenic, eicosapentaenoic and docosahexaenoic acids on hepatic lipogenesis and β-oxidation in rats. *Biosci. Biotechnol. Biochem.* **62**, 675-680.
11. Harris, W.S. (1989) Fish oil and plasma lipid and lipoprotein metabolism in humans: a critical review. *J. Lipid Res.* **30**, 785-807.
12. Cha, J.-Y., Y. Mameda, K. Oogami, K. Yamamoto and T. Yanagita (1998) Association between hepatic triacylglycerol accumulation induced by administering orotic acid and enhanced phosphatidate phosphohydrolase activity in rats. *Biosci. Biotechnol. Biochem.* **62**, 508-513.
13. Wong, S. H., P. J. Nestel, R. P. Trimble, G. B. Storer, R. J. Illman, and D. L. Topping (1984) The adaptive effects of dietary fish and safflower oil on lipid and lipoprotein metabolism in perfused rat liver. *Biochim. Biophys. Acta* **792**, 103-109.
14. Grundy, S. M., and M. A. Denke (1990) Dietary influences on serum lipids and lipoproteins. *J. Lipid Res.* **31**, 1149-1172.
15. Brinton, E. A., S. Eisenberg, and J. L. Breslow (1991) Increased apo A-I and apo A-II fractional catabolic rate in patients with low high density lipoprotein cholesterol levels with or without hypertriglyceridemia. *J. Clin. Invest.* **87**, 536-544
16. Hargreaves, K. M., D. J. Pehowich, and M. T. Clandinin (1989) Effect of dietary lipid composition on rat liver microsomal phosphatidylcholine synthesis. *J. Nutr.* **119**, 344-348.
17. Fukushima, M., S. Akiba, and M. Nakano (1996) Comparative hypocholesterolemic effects of six vegetable oils in cholesterol-fed rat. *Lipids* **31**, 415-419.
18. Huang, Y.S., M.S. Manhu, and D.F. Horrobin (1984) The effects of dietary cholesterol on blood and liver polyunsaturated fatty acids and on plasma cholesterol in rats fed various types of fatty acid diet. *Lipids* **19**, 664-672.
19. American Institute of Nutrition (1977) Report of the American Institute of Nutrition ad hoc Committee on standards for nutritional studies. *J. Nutr.* **107**, 1340-1348.
20. Folch, J., M. Lees, and G. H. Sloane-Stanley (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**, 497-506.
21. Sperry, W. M. and M. Webb (1950) A revision of the Shoenheimer-Sperry method for cholesterol determination. *J. Biol. Chem.* **187**, 97-106.
22. Fletcher, M. J. (1968) A colorimetric method for estimating serum triglyceride. *Clin. Chim. Acta* **22**, 393-397.
23. Rouser, G., A. N. Siakotos, and S. F. Fleischer (1966) Quantitative analysis of phospholipids by thin layer chromatography and phosphorus analysis of spots. *Lipids* 1, 85-86.
24. Yanagita, T., M. Satoh, N. Enomoto, and M. Sugano (1984) Di(2-ethylhexyl) phalate enhances hepatic phospholipid synthesis in rats. *Biochim. Biophys. Acta* **919**, 64-70.

25. Yanagita, T., K. Kobayashi, and N. Enomoto (1978) Accumulation of hepatic phopsholipids in rats fed di-2-ethylhexyl phthalate. *Biochem. Pharmacol.* **27**, 2283-2288.
26. Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. L. Randall (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265-275.
27. Wright, P. S., J. N. Morand, and C. Kent (1985) Regulation of phosphatidylcholine biosynthesis in Chinese hamster ovary cells by reversible membrane association of CTP: phosphocholine cytidylyltransferase. *J. Biol. Chem.* **260**, 7919-7926.
28. Ishidate, K., K. Iida, K. Tadokoro, and Y. Nakazawa (1985) Evidence for the existence of multiple forms of choline (ethanolamine) kinase in rat tissues. *Biochim. Biophys. Acta* **833**, 1-8.
29. Duncan, D. B. (1957) Mutiple range test for correlated and heteroscedastic means. *Biometrics* **13**, 164-176.
30. Lie, C. H., M. T. Huang, and P. C. Huang (1995) Sources of triglyceride accumulation in liver of rats fed a cholesterol-supplemented diet. *Lipids* **30**, 527-531.
31. Pelech S. L., and D. E. Vance (1989) Signal transduction via phosphatidylcholine cycles. *Trends Biochem. Sci.* **14**, 287-293.
32. Sundler, R., and B. Akesson (1975) Regulation of phospholipid biosynthesis in isolated rat hepatocytes. *J. Biol. Chem.* **250**, 3359-3367.
33. Vance, D. E. (1989) Regulatory and functional aspects of phosphatidylcholine metabolism. In Phosphatidylcholine Metabolism. D. E. Vance CRC Press, Inc. Boca Raton, Florida, FL. 225-239.
34. Asiedu, D., J. Skorve, A. Demoz, N. Willunsen, and R. K. Berge (1992) Relationship between translocation of long-chain acyl-CoA hydrolase, phosphatidate phosphohydrolase and CTP: phosphocholine cytidyltransferase and the synthesis of triglyceride and phosphatidylcholine in rat liver. *Lipids* **27**, 241-247.
35. Feldman, D. A., M. E. Rounsifer, and P. A. Weinhold (1985) The stimulation and binding of CTP: phosphocholine cytidylyltrasferase by phosphatidylcholine-oleic acid vesicles. *Biochim. Biophys. Acta* **929**, 429-437.
36. Surette, M. E., J. Whelan, G. L. Lu, K. S. Broughton, and J.E. Kinsella (1992) Dependence on dietary cholesterol for n-3 polyunsaturated fatty acid-induced changes in plasma cholesterol in the Syrian hamster. *J. Lipid Res.* **33**, 263-271.

魚油又は牛脂を摂取したラットの肝臓ミクロソーム リン脂質代謝に及ぼすコレステロールの影響

車　載英・柳田　晃良
（食品栄養化学研究室）
平成10年9月7日　受理

摘　　要

魚油又は牛脂食へのコレステロール負荷が，ラットの肝臓リン脂質代謝に及ぼす影響について検討した．コレステロール無添加食では，牛脂群に比べて，魚油群で肝リン脂質量は高値を示したが，コレステロールおよびトリグリセリド濃度は著しい差異はなかった．血清各脂質濃度は魚油群で低値を示した．コレステロール負荷食では，コレステロール—無添加食に比べて，肝リン脂質量は低下し，コレステロールおよびトリグリセリド濃度は顕著に増加した．コレステロール負荷により，両群で肝ミクロソームの phosphatidylcholine 割合の低下が認められた．さらに，牛脂群では phosphatidylethanolamine の割合の増加が，魚油群では sphingomyelin と lysophosphatidylcholine の割合の増加が認められた．コレステロール—無添加食では，魚油群で phosphatidylcholine—ドコサヘキサエン酸の割合が高値を示し，牛脂群で phosphatidylcholine—アラキドン酸　の割合が低値を示した．コレステロール負荷食で，両群とも phosphatidylcholine—アラキドン酸の割合と phosphatidylethanolamine—ドコサヘキサエン酸の割合の増加が認められた．さらに，コレステロール—負荷食では両群とも，肝臓 phosphatidylcholine 生合成の律速酵素であるミクロソーム CTP: phosphorylcholine cytidylyltransferase (cytidylyltransferase)活性は上昇し，細胞質 cytidylyltransferase 活性は低下した．すなわち，コレステロール食による生体膜　phosphatidylcholine の低下に応答して，細胞質からミクロソーム膜への酵素転移を介した cytidylyltransferase の活性が誘起されていることが推察された．本研究において，食餌性コレステロールのリン脂質代謝に対する影響は食餌脂肪の飽和度の違いによって異なることが認められた．